ABSTRACT
A rapid, specific high-pressure liquid chromatographic assay was used to study the pharmacology of pentamethylmelamine in 21 patients (28 infusions) receiving 80 to 1500 mg/sq m. In patients with normal liver function, pentamethylmelamine was rapidly cleared from the plasma with a terminal half-life of 2.2 hr. Abnormal liver function tended to correlate with increased half-life and reduced total clearance. In addition, increased neurological toxicity was associated with hepatic abnormalities. The N2,N2,N4,N6-tetramethylmelamine, N2,N4, N6-trimethylmelamine, dimethylmelamine, and monomethylmelamine metabolites were detected in plasma. The terminal plasma half-lives of these metabolites increased with decreasing number of methyl group. With liver dysfunction, the plasma clearance of these metabolites also decreased and central nervous system toxicity increased. Although the antitumor activity of pentamethylmelamine is thought to be mediated by the intermediate hydroxymethyl metabolites produced by hepatic microsomal oxidation or by the formaldehyde generated, the neurological toxicity appears to depend upon the pharmacokinetics of the drug and its demethylated metabolites.

INTRODUCTION
PMM is an antitumor agent undergoing clinical trial. It is a demethylated derivative of HMM, an agent that is clinically active against a number of solid tumors including ovarian cancer, lung cancer, and breast cancer. However, HMM is only sparingly aqueous soluble, making it unsuitable for parenteral administration. PMM, which has activity similar to that of HMM against a number of animal tumors, is sufficiently aqueous soluble for parenteral administration.
PMM and HMM are considered to be alkylating agents requiring metabolic activation (8). They are not active against cells in culture, but in the presence of liver microsomal enzymes they are cytotoxic (8). In vivo, both agents are extensively demethylated (10, 11). Presumably, demethylation is mediated by the hepatic mixed-function oxidases, with the formation of an intermediate hydroxymethyl product (Chart 1). This intermediate, which is cytotoxic in vitro, can spontaneously degrade to give the demethylated melamine (2,2,4,6-TEMM, in the case depicted in Chart 1) and formaldehyde. Formaldehyde is cytotoxic (7). Each of the methylated melamines can follow this same metabolic pathway. They all have in vivo antitumor activity, although activity decreases with decreasing number of methyl groups (5). The exact mechanism of action of the methylated melamines in unknown; however, using either ring-labeled or methyl-labeled HMM, radioactivity is found to be associated with macromolecules (9).
The pharmacology of PMM in 3 patients has been reported (1), showing a rapid terminal plasma half-life. Although the gas chromatographic assay used did detect the PMM demethylated metabolites, no pharmacokinetic data were presented. We developed a sensitive, specific, and efficient HPLC assay for PMM and its metabolites including the isomers of trimethylmelamine and tetramethylmelamine, but not DMM (2). We now report the pharmacology of PMM and its metabolites in 21 patients (28 infusions) receiving PMM (80 to 1500 mg/sq m).

MATERIALS AND METHODS
Chemicals. 2,2,4,6-TEMM, 2,2,4,4-TEMM, 2,2,4,6-TRMM, N2,N4-dimethylmelamine, and MMM were supplied by Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. N2, N4-Dimethylmelamine was supplied by Dr. D. E. V. Wilman, Chester Beatty Research Institute, London, England. Glass-distilled methanol for HPLC studies was obtained from Burdick and Jackson Labs., Inc. (Muskegon, Mich.). All other chemicals were obtained from regular commercial suppliers.
Patient Characteristics. Patients (21, receiving a total of 28 infusions) with advanced solid tumors were treated with PMM (80 to 1500 mg/sq m, once daily for 5 days) as part of the Phase 1 evaluation of PMM. Infusion duration was 2 to 4 hr. Hepatic and renal functions were determined by the SMA-12 analyzer. The normal ranges for liver enzyme activity and bilirubin are: alkaline phosphatase, 30 to 85 milliunits/ml; lactate dehydrogenase, 100 to 225 milliunits/ml; serum glutamate-oxalate transaminase, 10 to 50 milliunits/ml, total bilirubin, 0.15 to 1.0 mg/100 ml. All patients had normal renal function.
Sample Preparation. Blood was drawn into tubes containing heparin, and plasma was obtained by centrifugation. Urine was collected as voided and cerebrospinal fluid was obtained by lumbar puncture. Biological fluid (2 ml) was added to 3-ml Clin-Eut tubes (Analytichem International, Lawndale, Calif.). The tubes were eluted twice with 3 ml of ethyl acetate, with a 5-min delay between applications. The eluates were collected, combined, and evaporated with a stream of nitrogen. The residues were reconstituted with methanol (100 μl). Aliquots of 10 μl were analyzed by HPLC.
HPLC Analysis. Drug analyses were performed on a Waters Associates, Inc. (Milford, Mass.) liquid chromatograph.
equipped with a Model M6000 pump, a Model U6K injector, and a Model 440 UV detector, operating at 254 nm. Peak areas and retention times were determined by a Shimadzu Chromatopec-EIA electronic integrator. Separations were achieved on a C18-Bondapak column using 0.01 M ammonium formate, pH 3.5:methanol (60:40) as eluent at a flow rate of 1 ml/min.

**Pharmacokinetic Parameters.** Pharmacokinetic parameters were calculated using the NONLIN program developed at the Upjohn Company (6). The plasma clearances of PMM and appropriate metabolites were fit to the following biexponential nonlinear regression equation:

\[ C_p = A e^{-\alpha t} + B e^{-\beta t} \]

while the metabolites that had an initial increase of concentration were fit to the following equation:

\[ C_p = B' e^{-\beta t} - A' e^{-\alpha t} \]

\( C_p \) is the plasma concentration of PMM or a metabolite at time \( t \) after administration of i.v. drug, \( A \) and \( B \) are the intercepts at \( t = 0 \), and \( \alpha \) and \( \beta \) are the fast and slow disposition rate constants.

**RESULTS**

Chart 2 shows the plasma clearance of PMM and its metabolites in a patient with normal liver function after administration of PMM (1 g/sq m). PMM, 2,2,4,6-TEMM, and 2,4,6-TRMM are all rapidly cleared from the plasma, while DMM is much more slowly cleared with MMM increasing. In Chart 3 are shown the plasma clearances of PMM and its metabolites in a patient with abnormal liver function (bilirubin, 2 mg/100 ml) after PPM (640 mg/sq m). In this case, the clearance of PMM and those of its metabolites are considerably slower than in the previous case. Additionally, 2,2,4-TRMM is now observed and its plasma concentrations are still increasing at the end of the study. 2,2,4-TRMM was detected in the plasma of 3 patients, and 2,2,4,4-TEMM was detected in the plasma of only one patient.

The apparent dependence of PMM pharmacokinetics on the severity of liver disease is illustrated in Table 1. In addition, a possible correlation with CNS toxicity is observed. The major symptoms of CNS toxicity were agitation, confusion, and drowsiness. In 17 studies of patients with normal liver function, the mean terminal PMM plasma half-life was 2.2 hr. Only 4 of these studies were associated with CNS toxicity. In 5 studies involving patients with elevations of liver enzyme activities, the average PMM half-life was 2.5 hr. Although this is not signifi-

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**Clinical Pharmacology of PMM**

**Chart 1. Proposed mechanism of activation of PMM.**

**Chart 2. Plasma clearance of PMM and metabolites in a patient with normal liver function after PMM (1 g/sq m).**

**Chart 3. Plasma clearance of PMM and metabolites in a patient with abnormal liver function after PMM (640 mg/sq m).**
Table 1

Relationship of liver disease, PMM pharmacokinetics, and CNS toxicity

<table>
<thead>
<tr>
<th>Patients</th>
<th>$t_{1/2B}$ (hr)</th>
<th>Clearance (liters/hr)</th>
<th>CNS toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (17)*</td>
<td>2.2</td>
<td>182</td>
<td>4/17</td>
</tr>
<tr>
<td>Mild liver disease (5)</td>
<td>2.5</td>
<td>119</td>
<td>2/5</td>
</tr>
<tr>
<td>Moderate liver disease (3)</td>
<td>6.8</td>
<td>16.6</td>
<td>3/3</td>
</tr>
<tr>
<td>Severe liver disease (3)</td>
<td>9.2</td>
<td>15.4</td>
<td>3/3</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of studies.

Table 2

PMM metabolite pharmacokinetics

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>No. of cases</th>
<th>$t_{1/2}EL$ (hr)</th>
<th>$C \times t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,2,4,6-TEMM</td>
<td>11</td>
<td>4.3</td>
<td>37.3</td>
</tr>
<tr>
<td>2,2,4,4-TEMM</td>
<td>1</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2,4,6-TRMM</td>
<td>11</td>
<td>6.2 (8)</td>
<td>104.7 (8)</td>
</tr>
<tr>
<td>2,4,4-TRMM</td>
<td>3</td>
<td>3.8</td>
<td>17.8</td>
</tr>
<tr>
<td>DMM</td>
<td>9</td>
<td>14.6 (6)</td>
<td>117.7 (6)</td>
</tr>
<tr>
<td>MMM</td>
<td>9</td>
<td>31.7 (3)</td>
<td>628 (3)</td>
</tr>
</tbody>
</table>

* $t_{1/2} EL$, elimination half-life; $C$, concentration; $t$, time.

Moderate liver disease, i.e., elevation of liver enzyme activities and liver enlargement as determined by physical examination, the mean plasma PMM half-life was 6.8 hr, together with a drastic decrease in plasma clearance. In all of these studies, the patients experienced CNS toxicity. In one patient (studied 3 times) who had bilirubin of 2 mg/100 ml, the average PMM half-life was 9.2 hr and each infusion, although at decreased doses, resulted in CNS toxicity. Further, all patients with any degree of liver dysfunction receiving PMM doses greater than 640 mg/sq m experienced CNS toxicity.

In some cases, metabolite concentrations were still increasing at the end of the study; thus, elimination kinetics could not be determined. The observed elimination half-lives increased with decreasing number of methyl groups, except in the cases of the minor metabolites 2,2,4,6-TEMM and 2,2,4-TRMM (Table 2). The half-lives for these metabolites are much lower than expected. As with the PMM pharmacokinetics, the metabolite kinetics has a tendency to correlate to CNS toxicity. In 3 of 11 studies, the elimination half-lives of 2,4,6-TRMM could not be determined because of increasing metabolite concentrations; 2 of these 3 studies were associated with CNS toxicity. The 2 patients with toxicity also had liver dysfunction. In 3 of 9 studies, an elimination phase was not observed for DMM and 2 of the 3 were associated with CNS toxicity. In 3 of the 9 studies, an elimination phase was observed for MMM, and only one incidence of CNS toxicity was seen. CNS toxicity was observed in 5 of 6 of the remaining cases in which an elimination phase was not observed. It is noteworthy that the major constituents of the cerebrospinal fluid from patients receiving PMM were not PMM or tetramethylmelamine, but DMM and trimethylmelamine.

The urinary excretion of PMM and its metabolites was low. In 11 studies, the 24-hr cumulative excretion of PMM was only 0.75%, while total excretion was 7.8%. Chart 4 shows the 24-hr cumulative urinary excretion in a patient receiving PMM (1 g/sq m). The major excretory products were again DMM and 2,4,6-TRMM. In this patient, the minor metabolites 2,2,4-TRMM and 2,2,4-TEMM were also detected (not shown in Chart 4). Their excretion was of the same order of magnitude as that of PMM.

The Nash reagent was used to determine plasma formaldehyde concentrations. However, it was recently reported that this method detects not only formaldehyde but also the hydroxymethyl intermediates that give rise to formaldehyde (3). The average elimination half-life of the Nash reagent active species in 6 studies was 13.7 hr. This is less than the elimination half-lives of DMM and MMM.

**DISCUSSION**

The clinical usefulness of PMM is severely limited by CNS toxicity at doses that appear to be less than therapeutic (4). Our results suggest a relationship between incidence of CNS toxicity and plasma clearance of PMM and its metabolites. Since the urinary excretion of these materials is very low, it would appear that hepatic clearance is more important. On the other hand, the plasma clearance of formaldehyde is greater than that of DMM and MMM. This tends to indicate that renal clearance is more important for these 2 metabolites.

In those patients with liver dysfunction, plasma clearance was reduced and the incidence of CNS toxicity was greater. However, antitumor activity is thought to depend on hepatic metabolism. Thus, the mechanisms of CNS toxicity and therapeutic activity of PMM may be different. It may be then possible to ameliorate toxicity without affecting antitumor activity of PMM. This possibility is under investigation.

**ACKNOWLEDGMENTS**

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